the protein metabolism. The tumour cells do not proliferate at the expense of the tissues of the host, nor is there any evidence that they have a higher affinity for nutritive material than the growing cells of the host.

4. There is no evidence of the existence of substances secreted by the tumour disturbing the nitrogenous metabolism by means of a toxic action on the tissues of the host.

5. It is specially pointed out that these conclusions refer only to animals bearing tumours of sufficient size to warrant the assumption that they would reveal any specific property or function which may be possessed by the cells of a neoplasm. The effects which a large tumour must necessarily produce by virtue of its mere mass are not here considered.

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Contributions to the Biochemistry of Growth.—Distribution of Nitrogenous Substances in Tumour and Somatic Tissues.*

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In the preceding paper† a determination of the distribution of the nitrogen retained during a metabolism experiment showed that less nitrogen is needed to build up a given weight of tumour tissue than is necessary to build up an equal weight of the somatic tissues of the host. If this result is correct, it would follow that cancerous tissue should have a lower nitrogen percentage than the somatic tissues of the host.

We have therefore carried out nitrogen estimations of various tissues of Rats I, II, and III used in the experiments described in the preceding paper.

In order to make our results applicable to carcinomatous tumours, we examined the tissues of mice of about the same age, bearing a rapidly growing

* This research is in continuation of papers in 'Roy. Soc. Proc.,' B, vol. 80, 1908, p. 263, and this vol., p. 307, *supra*.

† W. Cramer and Harold Pringle, 'Roy. Soc. Proc.,' *supra*, p. 307.

adeno-carcinoma (Tumour B obtained from the Imperial Cancer Research Fund). Our results are given in Table I.

Table I.—Giving the Absolute Amounts of Total Nitrogen expressed in Percentage of Weight of Tissue, in the Various Tissues.

Animals.	Tumour.		Heart.		Muscle.		Liver.		Kidneys.	
	Amount of tissue used in grammes.	N per-centage.	Amount of tissue used in grammes.	N per-centage.	Amount of tissue used in grammes.	N per-centage.	Amount of tissue used in grammes.	N per-centage.	Amount of tissue used in grammes.	N per-centage.
Rat I	1·095	2·31	0·8040	3·04	0·3400	3·74	1·0310	3·04		
	1·025	2·47								
Rat II	0·2970	2·83	0·667	3·23	0·4900	3·63	0·5540	3·26		
	0·3230	2·77								
Rat III	No tu	mour	0·8400	3·06	0·3850	3·49	0·5720	3·08		
Mouse I ...	0·4134	2·27			0·3816	2·86	0·2900	3·14	0·1780	2·98
	0·4390	2·23			0·4262	2·69	0·3170	3·09		
Mouse II ...	0·5898	2·25					0·2946	2·75	0·2426	3·01
	0·7444	2·33					0·3240	2·80		
Necrotic part of same tumour	0·3193	2·37								
	0·3218	2·39								
Mouse III ...	0·7432	2·29			0·3073	2·94	0·3946	3·19	0·1869	3·29
	0·7700	2·29			0·2435	2·82	0·3686	3·19		

It will be seen that the amount of total N for the same tissues is remarkably constant for different animals of the same species. In every case the tumour tissue contains less nitrogen than the somatic tissues of the host. This is true both for the sarcomatous and for the carcinomatous tumours. In order to get an idea of the distribution of the nitrogenous substances, we have divided them into (*a*) those which are coagulated by boiling alcohol, and (*b*) those which are not coagulated. The latter do not give the biuret test. These two groups may be taken to correspond roughly with native proteins on the one hand and simpler abiuret substances on the other.

The determination of the relative amounts of coagulable and incoagulable nitrogenous material was carried out by the method which we have used in previous investigations.* This method we have found to give uniform and reliable results, and to be well adapted for the purpose of determining the

* Harold Pringle and W. Cramer, 'Journal of Physiology,' vol. 37, p. 2, 1908.

distribution of coagulable protein material in the various tissues. For this investigation we used the tissues of Rats I, II, and III, as well as those of a normal rat and of two rats, X and Y, of about the same weight, bearing very large tumours also derived from the same transplantable rat sarcoma (J. R. S.). The tumours of Rats X and Y were so large (30 grammes and 54 grammes respectively), that they had begun to grow at the expense of the tissues of the host. Rat Y was emaciated when killed. We also analysed the tissues of two mice which had carcinomatous tumours of medium size. The amount of tissue available from these latter being small, the tissues from the two were pooled and analysed together, and so the figures are the average for two. All the animals were kept on the same diet, viz., bread and milk.

The following table gives the result of the analyses of various tissues, the amounts of coagulable and incoagulable N are given in percentage of total N, and the figures indicate therefore the relative proportions in which the two groups of substances referred to above occur in the various tissues.

Table II.—Giving Proportion of Coagulable and Incoagulable Nitrogen expressed in Percentage of Total Nitrogen present in the Various Tissues.

Animal.	Tumour.		Kidney.		Liver.		Muscle.	
	Incoag. N percentage.	Coag. N percentage.	Incoag. N percentage.	Coag. N percentage.	Incoag. N percentage.	Coag. N percentage.	Incoag. N percentage.	Coag. N percentage.
Rat I	19·73	80·27	13·80	86·20	9·90	90·10		
Rat II	20·64	79·36	11·85	88·15	9·90	90·10		
Rat III	No tumour		14·10	85·90	10·40	89·60		
Rat X	15·70	84·30	17·97	82·03	13·83	86·17		
Rat Y	18·74	81·26	14·37	85·63	14·20	85·80		
Normal Rat Z ...	No tumour		14·37	85·63	11·60	88·40		
2 mice (carcinoma)	23·00	77·00	—	—	17·50	82·50	16·00	84·00

Referring to Table II, it will be seen that the analyses of the tissues of Rats I and II which had small tumours agree together; and that the analyses of the somatic tissues of these also agree with those of Rats III and Z, which were normal animals. Rats X and Y, which had large tumours, begin to show anomalies, particularly the kidney of Rat X, where the proportion of coagulable to incoagulable nitrogen deviates markedly from the normal.

There is a marked difference in the relative amount of coagulable nitrogen in the different tissues examined, and it will be seen that, with the exception

of Rat X, the tumour tissue in every case had a considerably smaller percentage of coagulable nitrogen than the somatic tissues, liver, kidney, or muscle (in the case of the mice).

The average figures for the percentage of coagulable nitrogen are as follows:—

	Tumour.	Kidney.	Liver.	Muscle.
Rats with tumours	81·3	85·50	88·04	
Rats, normal	—	85·76	89·0	
Mice with tumours.....	77·0	—	82·5	84·0

It will be seen that the proportion between coagulable and incoagulable nitrogen in tumour tissue and in somatic tissue is different in such a way that in the case of the rats the relative amount of the coagulable nitrogen of the tumour tissue is 4·2 per cent. less than in kidney, and 6·7 per cent. less than in liver. In the case of the mice it is 5·5 per cent. less than in the liver and 7 per cent. less than in muscle.

In order to determine the absolute amounts of coagulable and incoagulable nitrogen it is necessary to correlate these figures with the absolute amounts of total nitrogen in the liver and the tumour* which are available in the case of Rats I, II, and III from the protocol of the preceding paper.† In the case of Rats X and Y, no determinations of the absolute nitrogen-content of the various tissues were made, and as the stage of tumour growth was different in the two groups, we have thought it better not to make the results obtained from Rats I and II applicable to Rats X and Y. In the case of the mice, the small quantity of tissue available made it impossible to make estimations of absolute amount of nitrogen and also of the coagulable and incoagulable fractions in the same animal. We have therefore calculated our results in the case of the mice by correlating our estimations of the total nitrogen given in Table I with the figures giving the relative quantities of coagulable and incoagulable nitrogen as given in Table II.

Table III gives the absolute amount of coagulable and incoagulable nitrogen calculated from our data as above.

The agreement of the figures obtained for the absolute amounts of coagulable and incoagulable nitrogen in the somatic tissues obtained in the series of Rats I, II, and III by individual estimations is striking, and affords

* It was impossible to carry out duplicate determinations of total nitrogen, coagulable and incoagulable nitrogen, in the same animal in the case of other tissues owing to the small amount available.

† W. Cramer and Harold Pringle, 'Roy. Soc. Proc.,' *supra*, p. 307.

Table III.—Giving the Absolute Amounts of Total Nitrogen, Coagulable Nitrogen, and Incoagulable Nitrogen, expressed in Percentage of the Weight of Tissue, in the Various Tissues.

Animal.	Tumour.			Liver.			Muscle.		
	Total N.	Coag. N.	Incoag. N.	Total N.	Coag. N.	Incoag. N.	Total N.	Coag. N.	Incoag. N.
Rat I	2·39	1·92	0·47	3·04	2·74	0·30			
Rat II	2·80	2·22	0·58	3·26	2·94	0·32			
Rat III	No	tumour		3·08	2·76	0·32			
Mouse I ...	2·25			3·12			2·77		
Mouse II ...	2·29 } 2·28	1·76	0·52	2·77 } 3·03	2·50	0·53	— } 2·83	2·38	0·45
Mouse III ...	2·29			3·19			2·88		

evidence of the reliability of the methods. The table shows that both for mice and rats, and for carcinomata and sarcomata, the tissues of rapidly proliferating malignant new growths show a marked diminution, amounting to about one-quarter, of the substances which can be coagulated by alcohol. The substances which are not coagulated by alcohol show a slight but distinct increase as compared with somatic tissue.

Summary.

The rapidly growing cells of a malignant new growth, and the cells of the animal bearing it, show a marked quantitative difference in their chemical composition. Weight for weight, the cancer cells contain only about three-fourths of the protein substances present in the tissues of the host. In other words, with the same amount of protein a bigger mass of tumour tissue than of host tissue can be built up. The simpler (abiuret) nitrogenous products of cell metabolism, however, are present in slightly greater amount in the cancerous tissue.

These results are important in themselves, for the light they throw upon the chemistry and the metabolism of the cancer cell. The interpretation of their bearing on the growth of cancerous tissue may only be attempted with caution. As regards the rapidity of growth, it is possible to formulate conclusions; since the tissue of a neoplasm can be built up with less protein than the same weight of host tissue, the former must grow more rapidly than the latter under circumstances where both are using up nitrogen for mere growth at the same rate. In order to explain the rapidity of growth, it is not necessary to assume that the cancer cells build up protein more rapidly in a given time than the cells of the host, since we have shown that the former

require less protein, and in the preceding paper we have been unable to find any evidence in favour of the assumption that tumour cells have a higher affinity for the material necessary for the building up of new tissue.

If the significance we attach to the relation between the diminished nitrogen-content and the rapidity of growth of cancerous tissue is justified, the same relation should hold good not only for the tissues of a malignant new growth but equally well for any other rapidly growing tissue. In a former paper* we have pointed out the similarity which exists between the growth of cancer and the growth of the fœtus, and, in fact, preliminary experiments by Dr. J. Lochhead have shown that the tissues of the fœtus have a lower nitrogen percentage than those of the maternal organism.

The observations are being extended to a series of transplantable tumours of all grades of rapidity of growth and varied degrees of histological differentiation.

The expenses of this research have been defrayed by a grant from the Moray Research Fund of the University of Edinburgh.

* Cramer, "The Gaseous Metabolism in Rats inoculated with Malignant New Growths," 'Third Scientific Report of the Imperial Cancer Research Fund,' 1908, p. 427.